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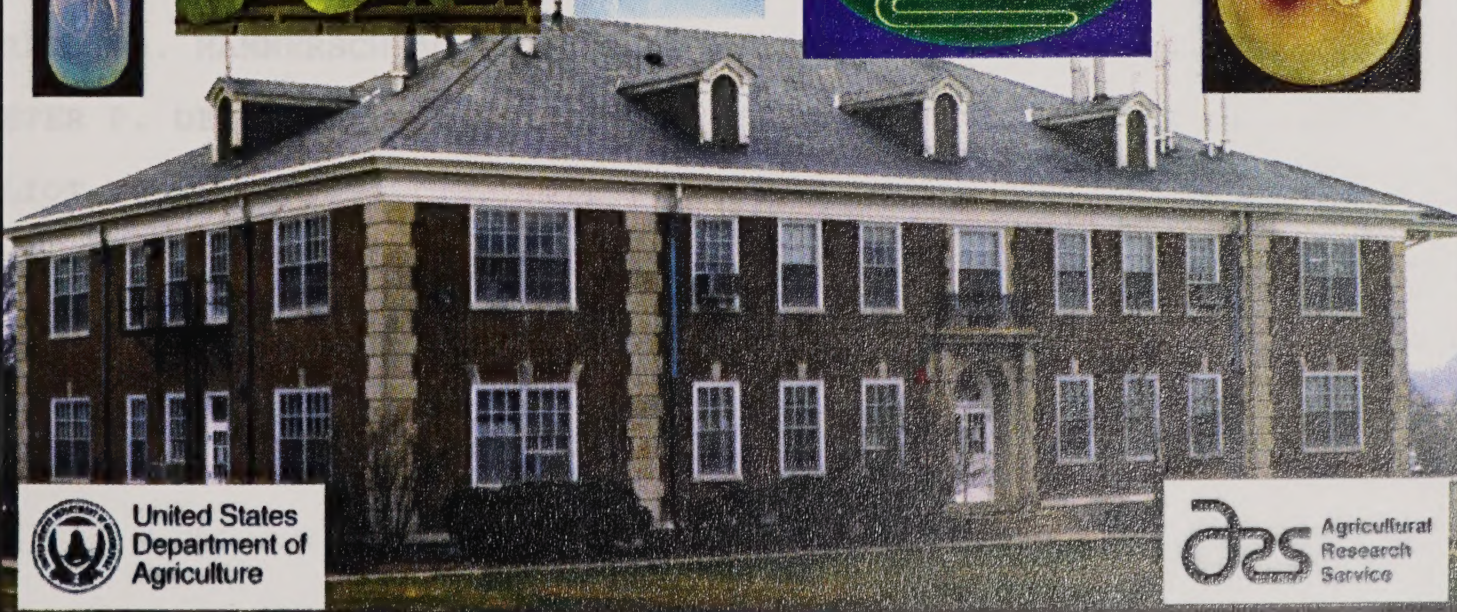
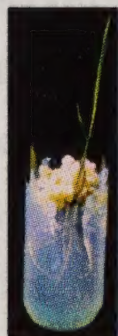
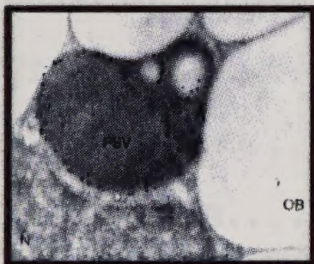
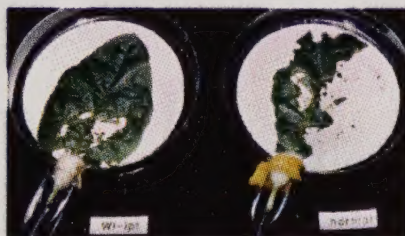
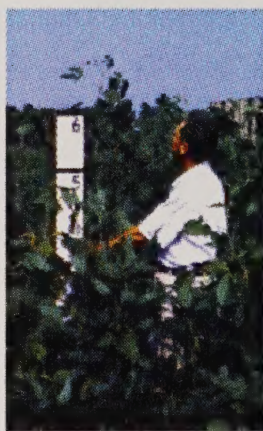
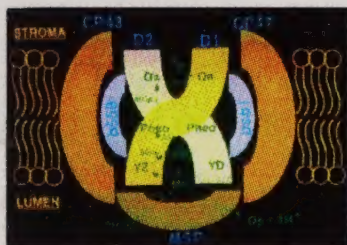
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PLANT MOLECULAR BIOLOGY LABORATORY

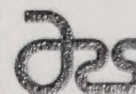
PLANT SCIENCES INSTITUTE

Brief Program Review
February 22, 1995

U.S.D.A., NAL
JUN 28 2001
Cataloging Prep



United States
Department of
Agriculture



Agricultural
Research
Service

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*Cover designed by Mark L. Tucker

AGENDA
Brief Review - Plant Molecular Biology Laboratory
Wednesday, February 22, 1995
Conference Room 021, Bldg. 005

| | | |
|-------|---|------------------------|
| 8:30 | Opening Remarks | Dr. R. F. Korcak |
| 8:35 | Introductions | Dr. R. F. Korcak |
| 8:40 | Comments | Dr. K. D. Murrell |
| 8:45 | PMBL Overview | Dr. A. K. Mattoo |
| 9:00 | Molecular Biology of Fruit Ripening | Dr. A. K. Mattoo |
| 9:15 | Molecular Regulation of Abscission | Dr. M. L. Tucker |
| 9:30 | Molecular Dissection of Essential Amino Acid Biosynthesis Genes | Dr. B. F. Matthews |
| 9:45 | Characteristics of the High-Lysine Producing Rice Mutant | Dr. G. W. Schaeffer |
| 10:00 | Break | |
| 10:15 | Mapping Agronomic Genes in Soybean | Dr. T. E. Devine |
| 10:30 | Identification of Genes Conferring Resistance to Cyst Nematode and <i>Phytophthora</i> in Soybean | Dr. B. F. Matthews |
| 10:40 | Gene Transfer of Anti-Pathogen Molecules in Soybeans and Sugarbeets | Dr. L. D. Owens |
| 10:55 | Developing Disease and Pest Resistance in Woody Crops | Dr. F. A. Hammerschlag |
| 11:10 | Molecular Approaches to Identify Fungal Resistance Gene(s) and <i>Septoria</i> Pathogens of Cereals | Dr. P. P. Ueng |
| 11:25 | Protein Targeting Mechanisms in Plants | Dr. E. M. Herman |
| 11:40 | Molecular Aspects of Cytokinin Action in Plants | Dr. A. C. Smigocki |
| 11:55 | Light-Mediated Regulation of Photosystem II Reaction Center Proteins | Dr. A. K. Mattoo |
| 12:05 | Executive Session | Dr. R. F. Korcak |

MISSION STATEMENTS

Research, Education, and Economics

The mission of Research, Education, and Economics is to create, apply, and transfer knowledge and technology to provide affordable food and fiber, ensure food safety and nutrition, protect the environment, and support the rural development and natural resource needs of people by conducting integrated national and international research, information, education, economic, and statistical programs and services that are in the national interest.

Plant Sciences Institute

The Plant Sciences Institute Research mission is to develop biological, chemical, and physical processes and principles including bioregulation that will improve pest management systems, improve crop production efficiency, improve conservation of natural resources, improve environmental quality, support regulatory and action agencies, and contribute to advances in biotechnology, and other societal benefits. The Institute's mission is accomplished through complex and exceptionally difficult fundamental and applied research programs in 16 laboratories.

Plant Molecular Biology Laboratory

The long-term goals of the Plant Molecular Biology Laboratory are to produce new crop plants that have higher nutritional quality, are environmentally compatible, have higher production efficiency, and are resistant to microbial diseases and pests. Toward those goals, PMBL scientists are unravelling basic molecular mechanisms involved in plant growth, development, and senescence. They are using combinatorial approaches of plant genetics, molecular biology, and tissue culture techniques for genetic improvement of soybean, wheat, rice, carrot, tomato, apple, peach and sugarbeets. PMBL scientists are developing and improving upon existing techniques for transferring genes into crop plants. Other research efforts are concentrated on identifying, isolating, and mapping agronomically important genes and studying their structure, organization, and regulation of expression. This research will enable growers to produce high-yielding and nutritious crops with low-input energy.

INTRODUCTION

The Plant Molecular Biology Laboratory (PMBL) is a focal point in ARS-USDA for the genetic engineering of crops. The focus is to produce value-added crops with better nutritional quality, better environmental compatibility, and greater resistance to diseases and pests. The molecular genetic approaches used in this program include identification, isolation, and redesigning of important and key plant genes, genetic transformation of agronomic crops with the designer genes and mass propagation of transgenic plants through successful regeneration of tissue cultured cells. The research outcome will enable farmers to produce safer, high-quality and well-defined nutritious crops with low input energy. Crops used are soybean, wheat, rice, peach, apple, sugarbeet, and tomato. Among the model plant systems used are *Arabidopsis*, duckweed, and carrot. The PMBL has state-of-the-art research facilities and provides the researchers the necessary equipment and environment to forge science ahead. Contributions made by the PMBL scientists are at the fore of molecular plant biology. The highly original, innovative, and productive research of PMBL scientists has been recognized nationally and internationally. Because of this high visibility, PMBL scientists have linkages with eight laboratories at BARC, six other ARS research locations, twenty-one universities in this country and twelve foreign countries.

The PMBL is housed in the first fully-renovated building, #006, at the Beltsville Center. The staff includes 10 permanent scientists, 7 visiting scientists, 13 postdoctoral research associates, 2 research assistants, 7 students, 6 biological technicians, 1 secretary, and 2 office automation assistants. The research team has a multidisciplinary base with expertise in plant genetics, biochemistry, physiology, pathology, molecular biology, and biotechnology.

RESEARCH ACCOMPLISHMENTS

Production of Disease Resistant Crops

The Problem:

Significant crop losses occur every year due to disease and pests. Control of pests through the use of pesticides, fungicides and insecticides is becoming increasingly unacceptable because of health risks and environmental concerns. Utilizing tissue culture and genetic engineering technologies to equip plants with new defense genes is a more attractive proposition, particularly in lowering needs for chemical pesticides.

Major Findings:

- High levels of resistance against bacterial leaf spot disease was demonstrated by peach somaclones under field conditions and by their progeny in the greenhouse.
- An in vitro micrografting technique, utilizing a digoxigenin (DIG)-labeled cRNA probe derived from Prunus necrotic ringspot virus (PNRSV) RNA3, was developed to monitor PNRSV in peach shoot cultures. This protocol will allow year-round rapid screening of peach shoots for resistance against PNRSV.
- Transgenic plants expressing a bacterial cytokinin biosynthesis gene were found to have a prolonged life span and improved resistance against tobacco (tomato) hornworm and green peach aphid.
- Developed model transgenic tobacco lines harboring a bactericidal peptide, cecropin-MB39, which display resistance to bacterial wildfire disease.
- Studies on the effects of cecropin-B on peach protoplasts, cells and intact tissues have demonstrated the feasibility of introducing the gene for this peptide into peach to enhance resistance against bacterial pathogens.
- Designed, constructed, and introduced into tobacco several constructs of the thionin gene, an antifungal barley gene.
- Generated DNA probes to distinguish between host specificity of Septoria diseases of wheat and barley. Using this molecular technique, we were able to show that barley-type S. nodorum is not phylogenically related to the wheat-type fungus, nor can it attack wheat. Diagnostic tools utilizing this information should help in diagnosis of early infection in wheat/barley plantlets.

Mapping Soybean Nuclear Genome and Producing New Soybean Germplasm

The Problem:

A comprehensive genetic map of soybean showing the location of the chromosomes of agronomically important genes would be of great value to soybean breeders. Further, it is important to know the exact location of genes on chromosomes to allow their identi-

fication, isolation and re-insertion for better utilization in improving crop plants.

Major Findings:

- Released 146 recombinant inbred maplines (SBML 1) of soybean germplasm. These lines were developed by eight generations of inbreeding and approach homozygosity for most traits. Homozygous lines retain genetic stability in successive generations and can be propagated indefinitely with genetic integrity.
- Mapped the local region surrounding the locus encoding a gene (Rhg4) responsible for resistance to soybean cyst nematode.
- Exceptionally tall and vigorous soybean lines were bred for silage. One line produced grain yield equal or better than the adapted grain-type soybean cultivars. In preliminary observations, the green manure-mulch crop of silage soybeans was effective in increasing the yield of cauliflower.

Enhancing Nutritionally-Important Amino acids in Plants

The Problem:

Plants are deficient in essential amino acids. If we are to increase the quality of food for human and animal consumption it is necessary to elevate the content of nutritionally important amino acids, lysine, threonine and methionine.

Major Findings:

- Registered five germplasm lines of rice with enhanced lysine content. Discovered mutant rice lines whose gene transcripts show enhanced translation in vitro using heterologous cell-free systems.
- Identified, cloned, sequenced and expressed genes encoding key enzymes in lysine/threonine/methionine pathway, i.e., aspartate aminotransferases, dihydrodipicolinic acid synthase, aspartokinase-homoserine dehydrogenase and asparagine synthetase, in soybean and carrot.
- Constructed four genetically engineered alterations in the soybean gene encoding dihydrodipicolinic acid synthase to produce enzyme lacking feedback inhibition by lysine.

Controlling Post-Harvest Quality and Abscission of Plant Organs

The Problem:

Postharvest losses of horticultural crops due to physical damage, chemical injury and pathological decay are high enough to merit re-evaluation in terms of newer methods and technologies. One major plant hormone implicated in controlling the postharvest quality of horticultural crops is the simple gaseous hydrocarbon, ethylene. To improve quality and shelf life of produce it is essential to decipher the intricate mechanisms regulating this plant hormone. Genetic modification to regulate the production or

action of ethylene should provide precise control of quality, abscission, and postharvest shelf-life of fruits, vegetables and flowers.

Major Findings:

- Discovered a novel molecular regulation of expression of a key protein in ethylene biosynthesis, ACC synthase. We demonstrated that truncation of ACC synthase at the carboxy-terminal region results in the production of a hyper-active, monomeric and catalytically-more efficient ACC synthase. We are now tracing the fate and function of the C-terminal peptide fragments that are released upon specific proteolysis of ACC synthase. These peptide fragments when infiltrated into green tomato fruit regulate ripening, suggesting autocatalytic control of ethylene production may involve breakdown of ACC synthase. The basic information obtained should help in producing transgenic tomatoes that have superior quality and extended shelf-life.
- Another approach to control fruit ripening is based on the finding of an inverse developmental relationship between the fruit's ability to produce ethylene and to accumulate polyamines. Based on this rationale we have successfully transformed tomato cells with a polyamine-biosynthesis gene-construct fused to a ripening promoter. Overproduction of polyamines by genetic introduction of 8-adenosylmethionine decarboxylase in tomato fruit has resulted in transgenic fruit with high carotene and longer shelf-life characteristics. This approach may result in fruit with better color, processing and flavor qualities.
- Identified cDNA and genomic clones for abscission-specific cellulases and polygalacturonases from soybean, bean and tomato.
- Characterized the promoter sequences of abscission cellulase. Analysis of this promoter should give the means to regulate abscission of flowers, fruits and leaves.
- Isolated an ETR1 homolog, the putative ethylene receptor, from tomato.
- Tomato transformation has become a routine procedure in PMBL, which should enable genetic introduction and testing of genes with potentials to alter and improve plant growth, development and senescence.

Regulation of Carbon Partitioning and Deciphering the Involvement of Cytokinins

The Problem:

The process of carbon partitioning enables movement and accumulation of assimilates (sugars) from source (leaf) to sink (roots, fruits, seeds) impacting plant productivity. Exogenous application of cytokinins to plants seems to cause increased flux of assimilates towards the sink sites. Genetically regulating the endogenous cytokinins in a cell- and tissue-specific manner is expected to influence carbon partitioning and improve plant

productivity.

Major Findings:

- Cytokinin gene constructs have been successfully transformed into model plants that are being analyzed for sugar accumulation in roots, fruits and seeds. Since the phytohormone status of a plant determines the developmental changes in the sugarbeet storage root, this approach should result in increasing the sugar content of the tap-root.
- Isolated numerous cytokinin-responsive genes to elucidate the cytokinin signal transduction pathway in plants.
- Successfully transformed sugarbeet plants, which should help in genetic engineering of this crop.

Delineation of Processes Regulating Seed Oil and Protein Accumulation in Soybean

The Problem:

The quality of oil and protein content in soybean seed is essential to developing nutritious soybean protein supplement and soybean oil for human and animal consumption. A pre-requisite is to establish genetic mechanisms and identify genes that control the accumulation of protein and oil in soybean.

Major Findings:

- The accumulation of soybean seed protein and oil has been shown by soybean geneticists to be genetically linked. Our analysis of soybean genomic sequences has revealed that genes for storage proteins and oil body proteins share common controlling elements. Genetic engineering using these controlling elements will be useful in regulating the expression of fatty acid desaturases to modify the composition of soybean oil.
- A correlation was observed between mobilization of nitrogen from vegetative cells to the seed and the accumulation of a thiol protease transcript in vegetative cells, suggesting a linkage between the two.
- Identified the plant vacuole as the intracellular site for degradation of unstable bioengineered proteins.

Photosystem II Reaction Center Assembly & Turnover

The Problem:

The goal of this research is to elucidate fine molecular mechanisms by which light energy is converted to chemical energy and thence to food production in a process called photosynthesis.

Only 2% of sunlight gets converted to food, suggesting that the process of photosynthesis in plants is limited by some important enzymatic reactions. One limiting target has been identified as the photosystem II (PS-II) reaction center. PS-II reaction center is very unstable due to highly labile nature of one of its protein components, the D1 protein, which together with the D2 protein forms the PS-II reaction center heterodimer. To stabilize this reaction center and potentially improve photosynthetic efficiency, we have examined the molecular dynamics of the PS-II reaction center. Another related problem being addressed is the potential of the increased penetration of ultraviolet (UV_B) irradiation to the biosphere, due to anticipated thinning of the stratospheric ozone layer, on agricultural productivity and quality. To combat potential damage to crops it is important to identify, monitor and stabilize key cellular targets sensitive to UV_B irradiation.

Major Findings:

- Isolated and characterized an intact PSII reaction center particle from stromal membranes. Defined molecular details of the assembly and disassembly of PSII RC proteins.
- Discovered light-mediated phosphorylation and dephosphorylation of PS-II reaction center proteins. The actual function of this modification is not understood. We have approached this aspect of work by isolating, purifying and identifying the protein kinases responsible for phosphorylating the D1 and D2 proteins. We have made significant progress towards this direction, which will enable producing transgenic plants in which this gene is silenced to ascertain the function of phosphorylation. This research is important for understanding how plants utilize sunlight and in planning genetic engineering strategies to improve crop productivity and plant defense against environmental extremes.
- Discovered that environmentally-relevant UV_B irradiation damages photosynthesis by enhancing degradation of the heterodimer D1-D2. Knowledge derived from these studies will establish what composes a target for UV_B irradiation in plants and offer a valuable tool for evaluating crop sensitivity to UV_B stress. Investigations into the mechanisms of PSII protein damage by UV_B irradiation is relevant to anthropogenic changes in the environment and in developing UV_B resistant crops.

Major Technology Transfer Activities

Patents:

- 06/488,530: 'Plant transformation by gene transfer into pollen', J.A. Saunders & B.F. Matthews. 1993
- 07/746,705: 'Bifunctional protein from carrots (*Daucus carota*) with aspartokinase and homoserine dehydrogenase activities', B.F.

Matthews & J.M. Weismann. 1994

- 08/054,985: 'Enhanced insect resistance in plants genetically engineered with a plant hormone gene involved in cytokinin biosynthesis', A.C. Smigocki. Filed 1993.

Genes Isolated and Available to Scientific Community at Large:

- Tomato GRP, pT52, pT58, rRNA, invertase, ACC synthases, ACC oxidase, *ETR1* (putative ethylene receptor), abscission-specific polygalacturonase and cellulases, cytochrome P450, and S-adenosylmethionine synthetase. [12]
- Bean/soybean cellulases, aspartate aminotransferases, dihydrodipicolinic acid synthase, oleosin, thiol protease, BiP, asparagine synthetase, aspartokinase-homoserine dehydrogenase, and β -1,3-glucanase. [9]
- Carrot aspartate aminotransferase, and aspartokinase-homoserine dehydrogenase. [2]
- Wheat ACC synthase. [1]
- Tobacco cytochrome P450, S-adenosylmethionine synthetase, myosin, twitchin, and protein kinase C. [5]

CRADAS and Trustfunds

E. I. DuPont de Nemours and Co., "Genetic engineering of lysine synthesis in plants," B. F. Matthews.

PRODUCTIVITY SUMMARY
(Since Last Review, 1992)

Peer Reviewed Manuscripts Non-peer Reviewed

| CATEGORY 1 SY'S | FIRST AUTHOR | CO- AUTHOR | FIRST AUTHOR | CO- AUTHOR | PATENTS | TOTAL |
|------------------------|-----------------|---------------|-----------------|---------------|---------|-------|
| MATTOO | 1 | 16 | 3 | 7 | - | 27 |
| DEVINE | 7 | 4 | - | - | - | 11 |
| HAMMERSCHLAG | 2 | 11 | 2 | 2 | - | 17 |
| HERMAN | 2 | 10 | 3 | - | - | 15 |
| MATTHEWS | - | 9 | 3 | 5 | 2 | 19 |
| OWENS | 2 | 7 | 1 | - | - | 10 |
| SCHAEFFER | 3 | 1 | 1 | 2 | - | 7 |
| SMIGOCKI | 2 | 3 | 1 | - | 1 | 7 |
| TUCKER | - | 1 | 1 | - | - | 2 |
| UENG | 4 | 1 | - | 1 | - | 6 |
| COLLABORATIVE | | | | | | |
| MATTOO/ HERMAN | 1 | | | | | 1 |
| DEVINE/ MATTHEWS | 2 | | | | | 2 |
| HAMMERSCHLAG/ OWENS | 2 | | 1 | | | 3 |
| TOTALS | 28 | 63 | 16 | 17 | 3 | 127 |

*Average 4.23/SY/YEAR

*PMBL published an average of 42.3 publications per year

IN-HOUSE CRIS PROJECTS

CRIS: 1275-21000-060-00D Termination Date: 05/12/96 \$206,543
Title: Molecular Aspects of Photosystem II Reaction Center
Assembly & Turnover, in Particular of D1 Protein
SY: Autar K. Mattoo (0.5)

CRIS: 1275-21000-064-00D Termination Date: 04/22/96 \$289,708
Title: Isolation & Characterization of Gene(s) Regulating Lysine
& Protein Expression in Cereals (Rice)
SY: Gideon W. Schaeffer (1.0)

CRIS: 1275-21000-065-00D Termination Date: 05/01/95 \$204,919
Title: Molecular Mapping of the Soybean Nuclear Genome to Facili-
tate the Development of Improved Germplasm
SY: Thomas E. Devine (0.15) and Benjamin F. Matthews (0.25)

CRIS: 1275-21000-075-00D Termination Date: 05/28/97 \$198,233
Title: Genetic Engineering of Genes and Enzymes in a Pathway for
Nitrogen Distribution
SY: Benjamin F. Matthews (0.75)

CRIS: 1275-21000-077-00D Termination Date: 07/30/97 \$274,212
Title: Gene Transfer and Tissue Culture Technologies for Improve-
ment of Peach, Apple, Pear, and Soybean
SY: Freddi A. Hammerschlag (1.0) and Lowell D. Owens (0.1)

CRIS: 1275-21000-079-00D Termination Date: 09/30/97 \$565,555
Title: Gene Transfer to Sugarbeet for Improved Carbon Partition-
ing and Pest Resistance
SY: Lowell D. Owens (0.9) and Ann C. Smigocki (1.0)

CRIS: 1275-21220-085-00D Termination Date: 05/18/98 \$131,325
Title: Integrating Classical Genetics & Molecular Biology Ap-
proaches to Map the Soybean Genome
SY: Thomas E. Devine (0.85)

CRIS: 1275-21000-087-00D Termination Date: 06/26/99 \$446,841
Title: Engineering Tissue-Specific and Developmentally Regulated
Genes to Improve Soybean Yield and Quality
SY: Mark Tucker (1.0) and Autar K. Mattoo (0.25)

CRIS: 1275-21000-112-00D Termination Date: \$253,807
Title: Cellular and Molecular Aspects of Prompt and Adaptive
Responses to Environmental Stress in Wheat
SY: Eliot M. Herman (1.0) and Autar K. Mattoo (0.15)

**This is a new CRIS Project. A project statement is being written
at this time.**

CRIS: 1275-21220-023-00D Termination Date: 01/22/95 \$448,766
Title: Identification and Characterization of Disease Resistance
Genes and Gene Products of Wheat
SY: Peter P. Ueng (1.0) and Autar K. Mattoo (0.25)

OUTSIDE FUNDING

CRIS: 1275-21000-047-09R Termination Date: 08/02/97 \$24,300
Title: Recognition and Disposal of Misfolded Seed Proteins
SY: Eliot M. Herman

CRIS: 1275-21000-060-03T Termination Date: 05/12/96 \$10,000
Title: Environmental Stress and Photosynthesis
SY: Autar K. Mattoo

CRIS: 1275-21000-065-01R Termination Date: 09/30/95 \$52,261
Title: Construction of a Physical Map Encompassing Soybean Cyst
Nematode Resistance
SY: Benjamin F. Matthews

CRIS: 1275-21000-087-01T Termination Date: 08/31/95 \$108,000
Title: Molecular and Functional Analysis of Cellulase and Polyga-
lacturonase Gene Expression in Abscission
SY: Mark L. Tucker

SPECIFIC COOPERATIVE AGREEMENTS

CRIS: 0500-00040-001-25S Termination Date: 08/31/96
TITLE: Develop Resistance to Potato Virus
SY: Autar K. Mattoo ADODR, B. K. Iskakov

COOPERATING INSTITUTION: Institute of Molecular Biology and
Biochemistry, Kazakhstan

CRIS: 1275-21000-087-02S Termination Date: 06/30/95
Title: Ethylene and Stress Related Gene Expression in Soybean
SY: Autar K. Mattoo, Mark L. Tucker, Benjamin F. Matthews, and
Theophanes Solomos

COOPERATING INSTITUTION: University of Maryland, College Park

CRIS: 1275-21220-023-01S Termination Date: 05/30/96
Title: Molecular Biology of Disease and Stress Resistance in
Cereals
SY: Peter P. Ueng and Theophanes Solomos

COOPERATING INSTITUTION: University of Maryland, College Park

TRUST FUND COOPERATIVE AGREEMENT

No. 58-1275-4-067: E. I. DuPont
Title: Genetic Engineering of Lysine Synthesis in Plants
SY: Benjamin F. Matthews

Revised

FUTURE-YEAR PROJECTIONS

Institute: PSI
Date: 1/27/95

MU Name: Plant Molecular Biology Laboratory
Initials: RL _____ AO _____

| | FY 1995 | FY 1996 | FY 1997 |
|---|------------------|----------------|----------------|
| BASE FUNDS (Net to Location) | * \$3,019,909.00 | \$3,019,909.00 | \$3,019,909.00 |
| SALARIES (include WGI's, promotions, retiremts. replacements, merit pay, etc.) | \$1,770,574.00 | \$1,839,561.36 | \$1,913,143.81 |
| Explanation of Variance to ARMPS: | 4.00% | 4.00% | |
| <hr/> | | | |
| % assumed increase from '95-96 & from '96-97 | | | |
| RSA SALARIES AND UTILITIES (Only those Absolutely Necessary) | \$79,837.00 | \$79,837.00 | \$79,837.00 |
| INDIRECT RESEARCH COSTS | \$685,576.00 | \$719,854.80 | \$755,847.54 |
| Inflation rate used for IRC | 5.00% | 5.00% | |
| INTERNAL INDIRECT RESEARCH COSTS | \$0.00 | \$0.00 | \$0.00 |
| Inflation rate - to be provided by Management Unit | | | |
| OTHER FIXED COSTS | \$102,480.00 | \$107,604.00 | \$112,984.20 |
| Hazardous & Radiological Waste Disposal | \$5,500 | | |
| Biometrics | \$1,500 | | |
| Postage | \$5,000 | | |
| Greenhouse Surveillance | \$11,000 | | |
| Telephones | \$18,000 | | |
| Maintenance Contracts | \$55,580 | | |
| Gasoline Credit Card Purchases | \$500 | | |
| Occupational Health Maintenance | \$1,000 | | |
| Federal Express | \$2,000 | | |
| Other - Shops | \$2,400 | | |
| Inflation factor | 5.00% | 5.00% | |
| MANDATORY EXTRAMURAL AND OTHER DIRECTED TRANSFERS OF FUNDS (Mandated by Congress or NPS) | | | |
| BALANCE AVAILABLE (Discretionary) | \$381,442.00 | \$273,051.84 | \$158,096.45 |
| SY's | 11 | 11 | 11 |
| DISCRETIONARY \$'s PER SY | \$34,676.55 | \$24,822.89 | \$14,372.40 |
| Total Federal FTE's | 33.61 | 33.51 | 33.51 |

General Notes: * This report reflects the elimination of CRIS 047 and includes funds for former CRIS 1275-21000-080.
Use FY 1995 base funds for FY 1996 and FY 1997 (assume no increases).
Do not include soft funds or "0500" CRIS Units funds or costs unless approved by ID.
Proposals for resolving underfunding problems may be addressed briefly on the back of this form.
Any information you include will be restricted to use in the Area Director's office ONLY. Do not use solutions to performance problems to resolve funding problems.
If a retirement is going to be used to resolve funding shortages, what will be the backup plan should the retirement not occur?

Summary of Financial Resources
FY 1995

| <u>Item</u> | <u>Amount</u> |
|----------------------------|--------------------------|
| Total to Location | \$ 3,019,909.00 |
| Indirect Research Costs | \$ 685,576.00 |
| Other Fixed Costs | \$ 102,480.00 |
| Total Funds Available | \$ 2,231,853.00 |
| Salary (% of total) | \$ 1,850,411.00 (82.91%) |
| All other (% of total) | \$ 381,442.00 (17.09%) |
| Total Dollars per SY | \$ 202,895.72 |
| Discretionary Funds per SY | \$ 34,676.55 |

MANAGERIAL CONCERNS

The PMBL responded in good faith to IRC and salary increases without any budget increase by abolishing more than a dozen support (several postdoctoral associates, one secretary, and several student/technician slots) positions that existed at the time of the last lab review, severely impacting the programs of Drs. Mattoo, Matthews, Devine, and Herman. None-the-less, PMBL scientists have continued their productive research with strong will, dedication and collaborative efforts, in line with the saying: when things get tough, the tough get going! The Beltsville Area has to look deeper and seriously into the future outcome of less and less support to laboratory scientists, particularly those working in disciplines such as Molecular Biology and Biotechnology, which are labor intensive and incur high costs.

Dr. Devine's and Dr. Schaeffer's CRIS's are barely operational this fiscal year and will become untenable next fiscal year if no additional support money is made available. The salaries in these CRIS's amount to 74% and 73%, respectively, for permanent employees; there are no temporary positions in these CRIS's. The additional support money could be requested from NPS. Alternatively, consolidation of different CRIS's in the laboratory are in order. The problem arising out of the closure of Dr. Herman's CRIS has been discussed with the Institute Director and NPS leader. It has been decided to redirect Dr. Herman to work on Molecular Aspects of Temperature Stress in Wheat and the money for this CRIS will be transferred from that previously obligated to CRIS 1275-21000-080-00D. Dr. Herman has been asked to write a new Project Statement.

The PMBL has upgraded, with the help of the Beltsville Area, a Biotech Facility in Room 6 in the basement. The equipment in this facility includes peptide synthesizer, peptide sequencer, DNA synthesizer, and DNA sequencer. We are working out an infrastructure such that Beltsville Area and other ARS scientists can use this facility. A full-time support scientist (GS-09) will be required to run this facility. It would be fitting and easy on all if BA supports this position.

The acquisition of a Silicon Graphics Computer has eased the access of PMBL scientists to updated databases. However, it is essential to network the PMBL building to enable optimum use of this facility. We need guidance and assistance in networking different laboratories and the secretarial office. The PMBL has been asked by the BA to get the networking done with lab funds.

SAFETY AND HEALTH REPORT

At this time, the Safety and Health office has scheduled our inspection for May 1995.

RESPONSE TO RECOMMENDATIONS FROM PREVIOUS REVIEW

The comments of the reviewers were very complimentary to the science being done in the Plant Molecular Biology Laboratory. The reviewers were very impressed with the productivity and the recognition of the scientists, nationally and internationally. A few suggestions were made about the redirection of a couple of programs. Both the Research Leader of PMBL and the Institute Director of PSI disagreed with the suggested redirection and let the programs stay as they were.

PLANT MOLECULAR BIOLOGY LABORATORY - STAFFING CHART

Autar K. Mattoo
Research Leader

Angela Benjamin, Secretary
Audrey Lemucchi, Office Automation Clerk
Dorothy Roach, Office Automation Clerk

Isabelle Booijs, Research Associate
Kyu Chung, Research Associate
Roshni Mehta, Research Associate
W. Mark Swegle, Research Associate
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Michael Reinsel, Biol. Sci. Lab Tech (Biotech Facility)
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Peter U. Ueng
Plant Pathologist

Kuppuswamy Subramaniam, Research Associate
Lixin Wang, Visiting Scientist

I. Name: Autar K. Mattoo
Title: Research Leader & Supervisory Plant Physiologist

II. CRIS Project: 1275-21000-087-00D
**CRIS Title: ENGINEERING TISSUE-SPECIFIC AND
DEVELOPMENTALLY REGULATED GENES TO IMPROVE
SOYBEAN YIELD AND QUALITY**

Progress: We have discovered a novel molecular regulation of a wound-inducible and ripening-related ACC synthase, a key protein involved in the aging process (ripening and senescence) of higher plants. The carboxyl-terminal region of this enzyme was found to be involved in its dimerization and in regulating enzyme catalysis. Peptide fragments encompassing this C-terminal region were found to regulate the production of the ripening hormone ethylene and color development in tomatoes. We have isolated complementary and genomic sequences for ACC synthase from soybean. A novel wound-inducible ACC synthase gene was isolated and expressed in a functional form in *Escherichia coli*.

Plans: To test the mechanism of induction of ripening/senescence in plant tissues by the 'ripening-inducing' peptides and to generate transgenic plants transformed with synthetic gene(s) corresponding to the C-terminal fragments of ACC synthase. Other plans are to sort out the gene family of ACC synthase and ethylene-receptor in soybean.

CRIS Project No: 1275-21220-023-00D
**CRIS Title: IDENTIFICATION AND CHARACTERIZATION OF
DISEASE RESISTANCE GENES AND GENE PRODUCTS
OF WHEAT**

Progress: Several different host defense-related genes (glycine-rich protein, pT52, pT58) and gene-products (peptidyl prolyl *cis trans* isomerase, serine carboxypeptidases) were isolated, identified, localized and characterized. The role of ethylene in elicitor-induced plant responses was studied using a model that involves interactions between the host leaf and a fungal xylanase. Ethylene induction under these conditions was found to involve differential accumulation of transcripts for ACC synthase and ACC oxidase, the two key enzymes in ethylene biosynthesis pathway.

Plans: We will apply our knowledge obtained with model plants to improve wheat crop. In addition, we will conduct additional studies on drought-tolerance and photoinactivation mechanisms that affect disease development and photosynthetic capacity of wheat leaf.

CRIS Project No: 1275-21000-060-00D
CRIS Title: MOLECULAR ASPECTS OF PHOTOSYSTEM II
REACTION CENTER ASSEMBLY AND TURNOVER, IN
PARTICULAR OF D1 PROTEIN

CRIS Project No: 1275-21000-060-02T
CRIS Title: UV-B RADIATION-INDUCED DAMAGE TO
PSII REACTION CENTER PROTEINS AND ADAPTIVE
PROTECTION

Progress: We have discovered light-mediated reversible phosphorylation of photosystem II (PS II) reaction center proteins and proposed its role as a sensing mechanism for the chloroplast to adapt to chromatic adaptation to light conditions. We further demonstrated that not only the rapidly-turning-over D1 protein but also its sister protein, D2, is a target of environmentally relevant ultraviolet-B irradiation damage, suggesting the D1-D2 heterodimer constituting the PS II reaction center is the real target of UV-B destabilization. Finally, successful isolation, identification and characterization of a PS II reaction center particle from stroma lamellae has provided strong evidence that, even though PS II is concentrated and functional in grana membranes, PS II reaction center is assembled in stroma lamellae and then translocated to grana.

Plans: We are isolating the protein kinase that phosphorylates the D1 protein. We will clone this gene, express it in an opposite orientation to silence the D1-kinase, and then test the role of D1 phosphorylation in the dynamics of D1-D2 metabolism and function. We will extend our observation of D1-D2 heterodimer as a protein target of UV-B and test its relevance to agronomic crops using UV-B sensitive and UV-B resistant lines of soybean. Long term goal is to transform agronomic crops (soybean and wheat) with flavonoid biosynthesis enzyme gene(s) attached to a UV-B and high light responsive promoters which should result in overproduction of sun-screen compounds and protect transgenic plants from high-light and UV-B irradiation damage.

III. Cooperators:

- J. D. Anderson, WSL, BARC
- H. Norman, WSL, BARC
- S. Dube, Univ of Md., College Park
- T. Solomos, Univ. of Md., College Park
- A. K. Handa, Purdue Univ., Indiana
- U. Feller, Univ of Berne, Switzerland
- M. T. Giardi, CNR, Roma-Via Salaria, Italy
- M. Edelman, Weizmann Inst., Israel
- B. B. Chattoo, Univ of Baroda, India
- S. K. Sopory, JN Univ., New Delhi, India

•B. K. Iskakov, Kazakhstan

IV. Publications (since last review)

Peer Reviewed

Raina, A. K., Kingan, T. G. and Mattoo, A. K. 1992. Chemical signals from host plant and sexual behavior in a moth. *Science* 255:592-594.

Elich, T. D., Edelman, M. and Mattoo, A. K. 1992. Identification, characterization and resolution of the *in vivo* phosphorylated form of the D1 photosystem II reaction center protein. *J. Biol. Chem.* 267:3523-29.

Mattoo, A. K., Mehta, R. A. and Baker, J. E. 1992. Copper-induced ethylene biosynthesis in terrestrial (*Nicotiana tabacum*) and aquatic (*Spirodela oligorrhiza*) higher plants. *Phytochemistry* 31:405-409.

Mehta, R. A., Fawcett, T. W., Porath, D. and Mattoo, A. K. 1992. Oxidative stress causes rapid membrane translocation and *in vivo* degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase. *J. Biol. Chem.* 267:2810-2816.

Li, N., Parsons, B. L., Liu, D. and Mattoo, A. K. 1992. Accumulation of wound-inducible ACC-synthase in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology* 18:477-487.

Li, N., Wiesman, Z., Liu, D. and Mattoo, A. K. 1992. A functional tomato ACC synthase expressed in *Escherichia coli* demonstrates suicidal inactivation by its substrate S-adenosylmethionine. *FEBS Letters* 306:103-107.

McCauley, S. W., Baronavski, A. P., Rice, J. K., Ghirardi, M. L. and Mattoo, A. K. 1992. A search for subpicosecond absorption components in photosystem II reaction centers. *Chemical Physics Letters* 198:437-442.

Breiman, A., Fawcett, T. W., Ghirardi, M. L. and Mattoo, A. K. 1992. Plant organelles contain distinct peptidyl prolyl *cis trans* isomerases. *J. Biol. Chem.* 267:21293-21296.

Bailey, B. A., Avni, A., Li, N., Mattoo, A. K. and Anderson, J. D. 1992. Nucleotide sequence of the *Nicotiana tabacum* cv. xanthi gene encoding 1-aminocyclopropane-1-carboxylate synthase. *Plant Physiol.* 100:1615-1616.

Ghirardi, M. L., Mahajan, S., Sopory, S. K., Edelman, M. and Mattoo, A. K. 1992. Photosystem II reaction center particle from *Spirodela stroma lamellae*. *J. Biol. Chem.* 268: 5357-5360.

Jansen, M. A. K., Mattoo, A. K., Malkin, S. and Edelman, M. 1992. Direct demonstration of binding-site competition between photosystem II inhibitors at the Q_B niche of the D1 protein. *Pesticide Biochem. Physiol.* 46: 78-83.

Elich, T. D., Edelman, M. and Mattoo, A. K. 1993. Dephosphorylation of photosystem II core proteins is light-regulated *in vivo*. *EMBO J.* 12: 4857-4862.

Liu, D., Li, N., Dube, S., Kalinski, A., Herman, E. and Mattoo, A. K. 1994. Molecular characterization of a rapidly and transiently wound-induced soybean (*Glycine max* L.) gene encoding 1-aminocyclopropane-1-carboxylate synthase. *Plant Cell Physiol.* 34: 1151-1157.

Parsons, B. L. and Mattoo, A. K. 1994. A wound-repressible glycine-rich protein transcript is enriched in vascular bundles of tomato fruit and stem. *Plant Cell Physiol.*, 35: 27-35.

Li, N. and Mattoo, A. K. 1994. Deletion of the carboxyl-terminal region of ACC synthase, a key protein in the biosynthesis of ethylene, results in catalytically hyper-active, monomeric enzyme. *J. Biol. Chem.* 269:6908-6917.

Avni, A., Bailey, B. A., Mattoo, A. K. and Anderson, J. D. 1994. Induction of ethylene biosynthesis in *Nicotiana tabacum* by a *Trichoderma viride* xylanase is correlated to the accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase transcripts. *Plant Physiol.*, 106: 1049-1055.

Zhou, D., Mattoo, A. K., Li, N., Imaseki, H., and Solomos, T. 1994. Complete nucleotide sequence of potato tuber acid invertase cDNA. *Plant Physiol.*, 106: 397-398.

Norman, H.A., Pillai, P., St. John, J.B. and Mattoo, A.K. 1994. *In vivo* and *in vitro* studies of chloroplast n-3 fatty acid desaturase activity in an *Arabidopsis* mutant deficient in polyunsaturated fatty acids. *Plant Physiol.*, in press.

Symposia, Book Chapters and Proceedings:

Elich, T. D., Edelman, M. and Mattoo, A. K. 1992. Redox-regulated protein phosphorylation and photosystem II function. In: *Regulation of Chloroplast Biogenesis*, (ed. J. H. Argyroudi-Akoyunoglou), Plenum Press, New York, pp. 533-537.

Jansen, M. A. K., Driesenaar, A. R. J., Kless, H., Malkin, S., Mattoo, A. K. and Edelman, M. 1992. PS-II inhibitor binding, Q_B -mediated electron flow and rapid degradation are separable properties of the D1 reaction centre protein. In: *Regulation of Chloroplast Biogenesis*, (ed. J. H. Argyroudi-Akoyunoglou),

Plenum, New York. pp. 295-304.

Sopory, S. K., Ghirardi, M. L., Greenberg, B. M., Elich, T., Edelman, M. and Mattoo, A. K. 1992. Regulation of the 32kDa-D1 photosystem II reaction center protein. In: *Photosynthesis: Photoreactions to Plant Productivity* (eds. Y. P. Abrol, P. Mohanty and Govindjee), Oxford/IBH, New Delhi, pp. 131-156.

Anderson, J. D., Bailey, B. A., Taylor, R., Avni, A., Sharon, A., Mattoo, A. K., and Fuchs, Y. 1992. The role of ethylene in elicitor-induced plant responses. *Transactions of the Malaysian Society of Plant Physiology*, 3:124-128.

Mattoo, A. K., Elich, T. D., Ghirardi, M. L., Callahan, F. E. and Edelman, M. 1993. Post-translational modification of chloroplast proteins and the regulation of protein turnover. In: *Post-translational modifications in Plants* (eds. N.H. Betty, H.G. Dickinson and A.M. Hetherington), Society of Experimental Biology, U.K., pp. 65-78.

Mattoo, A. K., Li, N. and Liu, D. 1993. Tomato ACC synthase: Regulation of gene expression and importance of the C-terminal region in enzyme activity. In: *Cellular and Molecular Aspects of the Plant Hormone Ethylene* (Pech, J.-C., Latche, A. and Bologue, C., eds.), Kluwer Acad. Publ., Dordrecht (the Netherlands), pp. 223-231.

Anderson, J. D., Bailey, B. A., Taylor, R., Sharon, A., Avni, A., Mattoo, A. K. and Fuchs, Y. 1993. Fungal xylanase elicits ethylene biosynthesis and other defense responses in tobacco. In: *Cellular and Molecular Aspects of the Plant Hormone Ethylene* (Pech, J.-C., Latche, A., and Balague, C., eds.), Kluwer Acad. Publ., Dordrecht (the Netherlands), pp. 197-204.

Jansen, M. A. K., Gaba, V., Greenberg, B., Mattoo, A. K. and Edelman, M. 1993. UV-B driven degradation of the D1 reaction-center protein of photosystem II proceeds via plastosemiquinone. In: *Photosynthesis Responses to the Environment, Current Topics in Plant Physiology*, vol. 8 (eds. H.Y. Yamamoto and C.M. Smith), American Society of Plant Physiologists, Maryland, pp. 142-149.

Mehta, R. A. and Mattoo, A. K. 1994. Gene expression and protein dynamics during tomato fruit ripening. *Proc. Intl. Postharvest Symposium, IHS*, in press.

Mattoo, A. K. 1994. Molecular strategies to enhance postharvest quality of horticultural crops - an overview. *Proc. Intl. Postharvest Symposium, IHS*, in press.

Manuscripts Submitted:

Mehta, R. A., Warmbardt, R. D., and Mattoo, A. K. Tomato leaf carboxypeptidase: identification, stress-regulation and specific localization in the paraveinal mesophyll vacuoles.

I. Name: Mark L. Tucker
Title: Molecular Biologist

II. CRIS Project: 1275-21000-087-00D
CRIS Title: ENGINEERING TISSUE-SPECIFIC AND
DEVELOPMENTALLY-REGULATED GENES TO IMPROVE
SOYBEAN YIELD AND QUALITY

CRIS Project: USDA/NRICGP 1275-21000-087-01T
CRIS Title: MOLECULAR AND FUNCTIONAL ANALYSIS OF
CELLULASE AND POLYGALACTURONASE GENE
EXPRESSION

Progress: The focus of this research program is on gene regulation during abscission (organ separation). We have prepared leaf abscission-specific cDNA libraries for bean, soybean and tomato. We identified clones for cellulases from bean, soybean and tomato and a polygalacturonases (PG) that is expressed in tomato leaf and flower abscission.

We have concentrated our efforts on analyzing the bean abscission cellulase promoter. In transgenic tomato, expression of a GUS reporter gene from a -210 bp bean promoter was transcriptionally regulated in tomato abscission zones; however, expression was not restricted to the leaf abscission zone but was found throughout the petiole. Transient expression assays using particle gun bombardment of bean showed that the bean cellulase promoter contains elements for tissue-specificity and hormonal regulation. We have partially shifted our focus to tomato so that we can begin study of an abscission promoter in its normal genetic environment in transgenic tomato. Northern blot analyses showed that transcripts for the tomato abscission PG are expressed in leaf and flower abscission zones and not in mature green or red ripe fruit. We recently identified cDNA clones for three abscission PGs and have identified genomic clones for both tomato abscission PGs and cellulase.

In addition to the above projects, Dr. Mattoo and I have initiated a project to study ethylene signal transduction in tomato abscission. From the tomato abscission cDNA library we identified a tomato homologue for the *Arabidopsis ETR1* gene. It has been suggested that the *ETR1* gene may code for the ethylene receptor. The tomato *ETR1* homologue is expressed constitutively in tomato abscission zones, stems, petioles, pedicles, and fruit.

Plans: The soybean and tomato abscission genes will be sequenced and compared to the bean abscission cellulase. This information will be used to identify conserved sequences that may act as cis-acting regulatory elements. This sequence information will aid in the design of constructs to be tested by transient expression and by stable transformation of tomato. In addition to the

promoter analyses, expression of tomato PG genes will be analyzed by RNase protection and *in situ* hybridization to determine temporal and spatial expression of these abscission genes. We will begin a study of the developmental regulation involved in cell commitment to abscission. As an initial step towards studying cell commitment to abscission we will transform tomato with a chimeric gene consisting of a PG promoter and GUS reporter gene. Expression of this chimeric gene will give a sensitive, cell-specific marker for cells induced to abscise. This will aid in our study of cell to cell signaling and cell commitment in abscission.

III. Cooperators

- Theophanes Solomos, Univ. of Md., College Park
- Roy Sexton, Stirling Univ., Scotland

IV. Publications (since last review):

Peer Reviewed

Kemmerer, E. C. and Tucker, M. L. 1994. Comparative Study of Cellulases Associated with Adventitious Root Initiation, Apical Buds, and Leaf Flower, and Pod Abscission Zones in Soybean. *Plant Physiol.* 104:557-562.

Symposia, Book Chapters, and Proceedings

Tucker, M. L., Matters, G. L., Koehler, S. M., Kemmerer, E. C., Baird, S. L., and Sexton, R. 1993. Hormonal and tissue-specific regulation of cellulase gene expression in abscission. In: J.C. Pech, A. Latche and C. Balague, *Proceedings of the International Symposium on Cellular and Molecular Aspects of the Plant Hormone Ethylene*. Kluwer Academic Publishers, Boston, pp. 265-271.

Manuscripts Submitted for Publication

Kalaitzis P., Koehler, S. M., and Tucker, M. L. 1994. Cloning of a tomato polygalacturonase and cellulase expressed in abscission. (Submitted)

I. Name: Benjamin F. Matthews
Title: Plant Biochemist

II. CRIS Project: 1275-21000-075-00D
CRIS Title: GENETIC ENGINEERING OF GENES AND ENZYMES IN
A PATHWAY FOR NITROGEN DISTRIBUTION

Progress: We identified carrot cDNAs encoding cytoplasmic aspartate aminotransferase (AAT) and aspartokinase-homoserine dehydrogenase (AK-HSDH). These were used to identify comparable soybean genes. From soybean, we identified three clones encoding AATs, the plastidic, mitochondrial and cytoplasmic isozymes, sequenced them, and functionally expressed them in *E. coli*. We purified the expressed soybean plastidic and mitochondrial enzymes from *E. coli* and raised antibody to them. We cloned two cDNAs encoding AK-HSDH, two encoding asparagine synthetase (AS), and one encoding dihydrodipicolinic acid synthase (DHPS). One AS and the DHPS cDNA were expressed functionally in *E. coli*. Alterations in the DHPS cDNA sequence resulted in DHPS that was much less sensitive to feedback inhibition by the end product, lysine, than the wild-type soybean DHPS. These altered cDNAs are being tested by a company to determine if they will be useful to increase the lysine content (nutritional value) of plants. The cDNA encoding AK-HSDH was used in a construct for *Arabidopsis* transformation. Several green plantlets exhibiting kanamycin resistance are being rooted and will be tested for the presence of functional soybean AK-HSDH. The promoter of one AK-HSDH was fused with GUS and transformed in tobacco. Surprisingly, floral tissue appears to express large amounts of AK-HSDH.

Plans: We will transform *Arabidopsis* with other cDNAs encoding the DHPS, AS and AAT. Combinations of different pathway genes will be made by crossing *Arabidopsis* plants to determine the effects of different genes and gene combinations on nitrogen flow and amino acid production. Promoters of these genes will be isolated from genomic clones. The promoters will be fused with the *UidA* gene encoding GUS for transformation of *Arabidopsis*. Enzyme expression and levels of pathway products will be monitored. We will identify promoter elements controlling tissue specific expression and regulation of each of these genes. Furthermore, we will engineer plants so they produce higher amounts of these essential amino acids and learn how to target pathway products.

CRIS Project: 1275-21000-065-00D
CRIS Title: MOLECULAR MAPPING OF THE SOYBEAN NUCLEAR
GENOME TO FACILITATE THE DEVELOPMENT OF
IMPROVED GERMPLASM

Progress: We are developing a linkage map of the soybean nuclear genome using restriction fragment length polymorphisms (RFLP),

known genes, randomly amplified polymorphic DNA (RAPD) and other techniques. Techniques are being developed for cloning genes by map position especially genes encoding soybean cyst nematode resistance.

Plans: We will continue to place known genes and other markers on the soybean map. We are developing a physical map of the local region surrounding the locus encoding a gene (Rhg4) conferring resistance to soybean cyst nematode race 3 via pulse field gel electrophoresis and other techniques.

III. Cooperators:

- Jim Saunders, CSL, Beltsville, MD
- Frank Turano, CSL, Beltsville, MD
- Dave Somers, University of Minnesota
- Burle Gengenbach, University of Minnesota
- Carl Falco, Dupont, DE
- Larry Beech, Pioneer, IA
- Cleo Hughes, Morgan State University
- Greg Wadsworth, Buffalo State University
- Michel Jacobs, Belgium
- Valerie Frankard, Belgium
- Gadi Galili, Israel

IV. Publications

Peer Reviewed (since last review):

Turano, F. J., Weisemann, J. M., and Matthews, B. F. 1992. Identification and expression of a cDNA clone encoding aspartate aminotransferase in carrot. *Plant Physiol.* 100:374-381.

Weisemann, J. M., Matthews, B. F., and Devine, T. E. 1992. A tight genetic linkage of the genes controlling seed coat color (I), soybean cyst nematode resistance (Rhg4), the 34 kD oil body protein and aspartokinase-homoserine dehydrogenase. *Theor. Appl. Genet.* 85:136-138.

Devine, T. E., Weisemann, J. M., and Matthews, B. F. 1993. Genetic linkage of the *Fr2* locus controlling root fluorescence and two RFLP markers. *Theor. Appl. Genet.* 85:921-925.

Wadsworth, G. J., Marmaras, S. M., and Matthews, B. F. 1993. Isolation and characterization of a soybean cDNA clone encoding the plastid form of aspartate aminotransferase. *Plant Molec. Biol.* 21:993-1009.

Lark, K. G., Weisemann, J. M., Matthews, B. F., Palmer, R. G., Chase, K., and Macalma, T. 1993. A genetic map of soybean (*Gly-*

cine max. L.) using an intraspecific cross of two cultivars: Minsoy and Noir1. Theor. Appl. Genet. 86:901-906.

Weisemann, J. M. and Matthews, B. F. 1993. Identification and expression of a cDNA from *Daucus carota* encoding a bifunctional aspartokinase-homoserine dehydrogenase. Plant Molec. Biol. 22:301-312.

Ghislain, M., Frankard, V., Vandenbossche, D., Matthews, B. F., and Jacobs, M. 1994. Molecular analysis of the aspartate kinase-homoserine dehydrogenase gene from *Arabidopsis thaliana*. Plant Molec. Biol. 24:835-851.

Muehlbauer, G. J., Somers, D. A., Matthews, B. F., and Gengenbach, B. G. 1994. Molecular genetics of the small gene family encoding the maize aspartate kinase-homoserine dehydrogenase bifunctional enzyme. Plant Physiol. 106:1303-1312.

Silk, G. W., Matthews, B. F., Somers, D. A., and Gengenbach, B. G. 1994. Cloning and expression of the DapA gene encoding dihydrodipicolinic acid synthase from soybean. Plant Molec. Biol. 26:989-993.

Smith, C. R., Saunders, J. A., Cheng, J., Van Wert, S., and Matthews, B. F. 1994. Expression of GUS and CAT activities in plants produced by pollen electrotransformation. Plant Science, 104:49-58.

Wadsworth, G. J., Gebhardt, J. S., and Matthews, B. F. 1995. Cloning and expression of the soybean gene encoding the mitochondrial form of aspartate aminotransferase. Plant Molec. Biol. (In Press)

Symposia, Book Chapters, Reviews:

Reardon, E. M. and Matthews, B. F. 1992. Penn State Symposium on Plant Biology: Biosynthesis and Molecular Biology of Amino Acids in Plants. Plant Molec. Biol. Reporter 10:294-298.

Saunders, J. A., Van Wert, S. L., Smith, C. R., Matthews, B. F., and Sinden, S. 1992. Successful gene transfer in plants using electroporation and electrofusion, In: Charge and Field Effects in Biosystems-3. M. J. Allen, S. F. Cleary, A. E. Sowers and D. D. Shillady (eds.), Boston Press, Boston, MA pp. 243-262.

Matthews, B. F. and Hughes, C. A. 1993. Nutritional improvement of the aspartate family of amino acids in edible crop plants. Amino Acids. 4:21-34.

Singh, B. K. and Matthews, B. F. 1994. Molecular regulation of amino acid biosynthesis. Amino Acids. 7:165-174.

Saunders, J. A., Smith, C. R., Cheng, J., Matthews, B. F., and O'Neill, N. R. 1994. Methods for gene transfer in plants using electrical manipulation. Book Chapter. International Technology Conference Proceedings, Malaysia. In press.

Matthews, B. F., Saunders, J. A., Gebhardt, J. S., Lin, J. J., and Koehler, S. 1995. Reporter genes and transient assays for plants. In: J. A. Nickoloff (Ed.) "Protocols for Electroporation and Electrofusion of Plant and Animal Cells" Vol __, Methods in Molecular Biology, Humana Press Inc., NJ, USA. In press.

Saunders, J. A. and Matthews, B. F. 1995. Pollen electrotransformation in tobacco. In: J. A. Nickoloff (Ed.) "Protocols for Electroporation and Electrofusion of Plant and Animal Cells" Vol __, Methods in Molecular Biology, Humana Press Inc., NJ, USA. In press.

Matthews, B. F., Hughes, C. A., Silk, G. W., and Gebhardt, J. S. 1994. Regulation of the aspartate family of amino acids in plants. In A. S. Basra (ed.) "Regulatory Mechanisms of Plant Metabolism." Germany. In press.

Patents:

06/488,530 "Plant transformation by gene transfer into pollen," James A. Saunders and Benjamin F. Matthews. Pending. License purchased by British Technology Group USA. 1993.

07/746,705 "Bifunctional protein from carrots (*Daucus carota*) with aspartokinase and homoserine dehydrogenase activities," Benjamin F. Matthews and Jane M. Weisemann. Allowed 1994.

- I. Name: Gideon W. Schaeffer
Title: Research Plant Physiologist/Biochemical Geneticist
- II. CRIS Project: 1275-21000-064-00D
CRIS Title: ISOLATION & CHARACTERIZATIONS OF GENE(S)
REGULATING LYSINE & PROTEIN EXPRESSION IN
CEREALS (RICE)

Progress: Purpose of this work is to characterize rice mutants and isolate genes associated with stress biochemistry and enhanced endosperm lysine. The research accomplished: A) Isolated and characterized unique proteins from mutant cell lines. B) Microsequenced two different peptides (nine and nineteen amino acids), searched the database and retrieved cDNA sequences assigned to rice tissue cultures. C) Germplasms derived from the mutant were registered, their seed was increased and packaged for distribution. D) Continued to establish relationships between glucanases and chitinases and the expression of the enhanced lysine phenotype in cell culture and the expression of these enzymes in seedlings and whole plants. Beneficiaries of this work include plant breeders, biochemists and molecular geneticists. New germplasm is now available for cultivar development with improved lysine. Molecular sequences are useful for biochemists and provide new tools for plant transformations and gene mapping projects.

Plans: The focus of future research is to: 1) identify factors that enhance *in vitro* translation of mutant mRNA (might be highly significant mutant for the modification of protein synthesis in cereals); 2) microsequence several unique seed storage proteins, identify them and study their function; 3) develop from our current rice mutants a transgenic rice line with sequences coding for feedback insensitivity for the lysine pathway, i.e. deregulation of dihydrodipicolinate synthase in embryogenic rice cultures (constructs are available from collaborators). This new line would be an altogether unique combination and potentially very useful.

III. Cooperators:

- R. Wu, Cornell Univ.
- M. Sorrels, rice database affiliations
- S. McCouch, rice database affiliations
- G. Gallili, Wieszmann Institute, Israel

IV. Publications (since last review)

Peer Reviewed

Schaeffer, G. W., Sharpe, Jr., F. T., and Dudley, J. T. 1992. Rice protein export mutant expression in liquid suspension cultures; Chitinases, β -glucanases and other proteins. Theor. Appl. Genet. 84:26-32.

Sharpe, F. T., and Schaeffer, G. W. 1993. Distribution of amino acids in bran, embryo and milled endosperm of in vitro-selected and lysine-enhanced mutant and wild type rice. Plant Science 90:145-154.

Schaeffer, G. W., Sharpe, F. T. and Dudley, J. T. 1994. Registration of five germplasm lines of rice selected in vitro for enhanced lysine. Crop Sci 34:1424-1425.

Schaeffer, G. W., Sharpe, F. T. Jr. and Dudley, J. T. 1994. Mutant of rice with enhanced lysine registered and evaluated for β 1-3 glucanase activity. Rice Biotechnology Quarterly 20:14-18.

Symposia, Book Chapter, and Proceedings

Schaeffer, G. W. and Sharpe, F. T. Jr. 1994. Shifts in storage protein subunits. Rice Biotechnology Quarterly 18:26-27.

Rutger, J. N. and Schaeffer, G. W. 1994. An environmentally sensitive genetic male sterile mutant of rice. Proceeding of Temperate Rice Conference, Australia.

Borojevic, K., Sesek, S. and Schaeffer, G. W. 1994. Efficiency of Another Culture Techniques in Wheat Breeding (In press, Susek, June 1994)

- I. Name:** Thomas E. Devine
Title: Research Geneticist
- II. CRIS Project:** 1275-21220-085-00D
CRIS Title: INTEGRATING CLASSICAL GENETICS AND MOLECULAR BIOLOGY APPROACHES TO MAP THE SOYBEAN GENOME

Objective 1: To integrate the classical genetic map of soybean with the emerging molecular marker map.

Progress: About 63 RFLP's and 28 RAPDs are segregating in a soybean cross along with morphological and disease resistance traits. 149 F3 progeny lines were classified for the genes controlling these traits and several genes have been mapped. The root fluorescence locus, Fr2, has been located in a catena of 4 RFLPs. The seed coat color locus, I, has been located proximal to two closely linked RFLP's; pBLT65, encoding aspartokinase-homoserine dehydrogenase, and pBLT24, encoding 34 kD thiol protease found in soybean seeds. The I locus is closely linked to the Rhg4 locus, controlling soybean cyst nematode. The proximity of these sequenced loci enhances the opportunity to identify and clone the Rhg4 locus, permitting the transfer of the Rhg4 gene to other crop cultivars and species. Further, the position of these loci provides the possibility of marker assisted selection as an alternative to selection by phyto-pathological assay. The SBML 1 germplasm, consisting of 146 recombinant inbred lines derived from the cross described above, was released to soybean researchers to facilitate mapping the soybean genome. These lines were developed by eight generations of inbreeding and approach homozygosity for most traits. Homozygous lines retain genetic stability in successive generations and can be propagated indefinitely with genetic integrity permitting researchers at other locations to contribute information on traits segregating in this cross to the genetic database.

Plans: In cooperation with Dr. B. F. Matthews of this laboratory, we will continue to analyze the DNA collected from F2 and F3 progeny and maplines from the cross PI290136 X BARC-2 (Rj4) with RFLPs and RAPDs in order to construct a more complete molecular map and to locate additional agronomically important genes in the genome map. Additional polymorphisms, for traits such as disease resistances, bacterial pustule, maturity genes and nutritional factors segregating in this cross will be sought and mapped. In addition, we will utilize selected molecular markers from the genetic maps of Dr. Shoemaker, ARS at Ames, Iowa and Dr. Lark, University of Utah, to integrate our molecular marker map with their maps. In cooperation with Dr. Matthews, we will use molecular markers to micro-map the region of the soybean genome proximal to the Rhg4 locus, which controls resistance to the soybean cyst nematode, with the ultimate objective of identifying and cloning the Rhg4 gene.

Objective 2: To expand and develop the classical genetic map of soybean.

Progress: The following gene pairs were tested for genetic linkage and found to segregate independently: W1 and Y10, Fr2 and W1, Y12 and W1, Y12 and Fr2, Dt1 and T, Dt1 and W1, L1 and T, L1 and W1, W1 and T, Dt1 and Ap, Dt1 and Ti, L1 and Ap, L1 and Ti, W1 and Ap, W1 and Ti, T and Ap, T and Ti, T and I, T and Rj4, I and Rj4, Y23 and Rj4, Fr2 and Pc, Fr2 and I, Pc and I, Df2 and F, Df2 and Rj1, Pc and Rj1, F and Pc, F and Y13, F and T, Rj1 and T, Rj1 and Y13, T and Y13, Rj2 and Y9, P1 and Y10, F and Y9, Pc and Y9, F and G, G and Rj1, F and I, G and I, I and Rj1, Fr1 and Y17, Fr2 and P2, Fr2 and W1, P2 and W1, P2 and Y17, Pc and Y10, Rj4 and Y17, Fr2 and Y13, and I and Rj2. This information is useful to soybean geneticists in differentiating linkage groups and constructing a comprehensive map of the soybean genome.

Plans: A comprehensive genetic map of soybean showing the location of the chromosomes of agronomically important genes would be of great value to soybean breeders. For example, the gene conditioning black seed coat color is known to be tightly linked (0.35% recombination) to the gene conditioning resistance to soybean cyst nematode. To obtain recombinant lines homozygous for nematode resistance but lacking the dark pigment would require only 1000 F₂ plants to yield 62 such individuals without genetic linkage. With a linkage of 0.35 GMU, however, 3 million F₂ plants would be needed to yield the desired recombinant plants. We will utilize progeny from a variety of crosses to expand the classical genetic map. Genes will be selected for testing using the following criteria: (a) clear unambiguous phenotypic expression, (b) expression of the trait in the seedling stage, (c) cost efficiency of evaluation, (d) availability of appropriate resources for assay of the trait, (e) agronomic value of the trait, (f) a probability assessment of the likelihood of linkage derived from computer analysis of published and unpublished linkage information.

Objective 3: To construct and evaluate soybean lines bred for use as a high protein silage for livestock.

Progress: Forty lines were bred for use as silage and evaluated at Beltsville, MD; Landisville, PA; Stuart's Draft and Mount Holly, VA; Ames and Jackson County, IA; and Lamberton and Waseca, MN. Eight promising lines were selected for advanced testing. Preliminary seed increases were obtained in Virginia for future testing and possible cultivar release. Forage yields of the silage lines exceeded those of conventional grain cultivars. Some silage lines harvested for grain may increase surface crop residue by as much as 41% over conventional grain type soybeans, demonstrating the potential to reduce soil erosion. Unexpected-

ly, one line bred for silage produced a grain yield equal or better than adapted grain type soybean cultivars. Preliminary research with Dr. Abdul-Baki indicated that a green manure-mulch crop of silage soybeans was effective in increasing yield of cauliflower.

Plans: The 8 silage lines selected will be evaluated in cooperation with ARS and State University scientists in Pennsylvania, Virginia, Iowa, Minnesota, New Hampshire and Arkansas for silage production and nutritional value. If the lines merit release as cultivars, production of breeders seed will be initiated. The silage line that showed good seed grain production will be evaluated for grain production in comparison with the best available cultivars. Should the data warrant, it may be released as a special purpose cultivar for soils particularly vulnerable to erosion. A silage line will be evaluated in cooperation with Dr. Abdul-Baki for use as a precursor crop to provide soil nitrogen and mulch cover for vegetable Brassica species. Additional crosses of the silage lines will be evaluated in Maryland, Pennsylvania, Virginia, and Minnesota to: (a) select new silage genotypes with improved disease resistance and superior vigor, (b) determine the inheritance of the vigor characteristics and (c) determine any possible linkages of the genes controlling vigor with disease susceptibility.

III. Cooperators:

- James E. McMurtrey, RSRL, BARC
- Aref Abdul-Baki, VL, BARC
- L. David Kuykendall, SARL, BARC
- C. D. Foy, CSL, BARC
- Randy Shoemaker, Iowa State University
- T. C. Kilen, Stoneville, Mississippi
- R. L. Bernard, University of Illinois
- Tadesse Mebrahtu, VSU, Virginia
- Dwayne R. Buxton, USDA, Ames, Iowa
- Elwood Hatley, Pennsylvania State University
- David Starner, Northern Piedmont Agricultural Extension Station, Virginia
- James Orff, University of Minnesota
- David Longer, University of Arkansas
- Richard Smith, University of Wisconsin
- Brad Hedges, Agriculture Canada Research Station, Ontario, Canada
- R.I. Buzzell, Agriculture Canada Research Station, Ontario, Canada

IV. Publications

Peer Reviewed (since last review):

Devine, T. E. and Kiang, Y. T. 1992. Test for linkage of the Ap and Ti loci in linkage group 9 with the Dt1 and L1 loci in linkage group 5. Soybean Genetics Newsletter 19:159-162.

Devine, T. E. 1992. Genetic independence of the W1 and Y10 loci and the Fr2, W1, and Y12 loci. Soybean Genetics Newsletter 19:163-165.

Devine, T. E. 1992. Genetic linkage tests for the Rj4 gene in soybean. Crop Science 32:961-964.

Foy, C. D., Duke, J. A., and Devine, T. E. 1992. Tolerance of soybean germplasm to an acid Tatum subsoil. Plant Nutrition 15:527-547.

Kuykendall, L. D., Saxena, B., Devine, T. E., and Udell, S. 1992. Genetic diversity in *Bradyrhizobium japonicum* and proposal for *Bradyrhizobium elkanii* sp. nov. Can. J. Microbiol. 38:501-505.

Weisemann, J. M., Matthews, B. F., and Devine, T. E. 1992. Molecular markers located proximal to the soybean cyst nematode resistance gene, Rhg4. Theor. App. Genet. 85:136-138.

Devine, T. E. 1992. Symposium on Crop Genome Mapping: Foreward. Crop Sci. 32:1085.

Devine, T. E., Weisemann, J. M., and Matthews, B. F. 1993. Linkage of the Fr2 locus controlling soybean root fluorescence and four loci detected by RFLP markers. Theor. App. Genet. 85:921-925.

Foy, C. D., Carter, Jr., T. E., Duke, J.A., and Devine, T.E. 1993. Correlation of shoot and root growth and its role in selecting for aluminum tolerance in soybean. J. Plant Nutrition. 16:305-325.

Devine, T. E., O'Neill, J. J., and Kuykendall, L. D. 1993. Near isogenic lines of soybeans as tools to identify nodulation specific-mutants of *Bradyrhizobium elkanii*. Plant and Soil 149:205-209.

Devine, T. E. and O'Neill, J. J. 1993. Genetic independence of the nodulation-response gene loci - Rj1, Rj2, and Rj4 - in soybean. J. Hered. 84:141-142.

Devine, T. E. and Kuykendall, L. D. 1994. Genetic allelism and linkage tests of a soybean gene, Rfg1, controlling nodulation with *Rhizobium fredii* strain USDA 205. Plant and Soil 158:47-51.

Foy, C. D., Lee, E. H., Shalunova, L. P., and Devine, T. E. 1994. Acid soil (aluminum) tolerance in soybean cultivars related to ozone tolerance. J. Plant Nutrition 18: (In press)

Manuscripts Submitted for Publication:

Kuykendall, L. D. and Devine, T. E. 1995. The lack of more severe rhizobitoxine chlorosis symptoms on the sensitive soybean genotype N53-3494 nodulated with Rj4-compatible mutants of *Bradyrhizobium elkanii* USDA61 (submitted to Plant and Soil).

I. Name: Lowell D. Owens
Title: Plant Physiologist

II. CRIS Project: 1275-21000-079-OOD
CRIS Title: GENE TRANSFER TO SUGARBEET FOR IMPROVED
CARBON PARTITIONING AND PEST RESISTANCE

Progress: 1) Developed model tobacco lines transgenic for cecropin-MB39 that display resistance to infection with *Pseudomonas syringae* pv. *tabaci*, the bacterial wildfire disease. 2) Introduced into tobacco several gene constructs encoding thionin, a fungitoxic polypeptide gene from barley. 3) Optimized conditions for transforming sugarbeet suspension cells by bombardment with DNA-coated particles. 4) Obtained a number of transgenic sugarbeet plants that express the β -glucuronidase (GUS) reporter gene.

Plans: 1) Further characterize the expression of the cecropin and thionin transgenes at the cellular level - especially as to whether the polypeptides are being secreted to the intracellular spaces as designed. 2) Determine the effectiveness of thionin-transgenic plants in protecting against fungal infection. 3) Determine whether the progeny of crosses between cecropin- and thionin-transgenic lines display a synergism in providing protection against plant pathogens. 4) Use the biolistic transient-transformation assay to examine the expression of various promoters in sugarbeet suspension cells in order to guide gene construction specifically for that crop. 5) Improve gene-transfer technology to sugarbeet to the point of being routine, and introduce the above two biocontrol genes.

III. Cooperators:

- Steve Sinden, VL, BARC
- Yong Huang and Jenifer McBeath, University of Alaska
- Chris Wozniak, NCSL, Fargo, ND

IV. Publications (since last review):

Peer Reviewed

Owens, L. D. and Eberts, D. R. 1992. Sugarbeet leaf disc culture: an improved procedure for inducing morphogenesis. *Plant Cell Tiss. Org. Cult.* 31:195-201.

Nordeen, R. O., Sinden, S. L., Jaynes, J. M., and Owens, L. D. 1992. Activity of cecropin SB37 against protoplasts from several plant species and their bacterial pathogens. *Plant Sci.* 82:101-

Owens, L. D. 1992. Letter to the Editor: Measurement of water availability in gel-solidified culture media. *Agricell Report*. 18:11

Hatfield, D., Choi, I. S., Mischke, S., and Owens, L. D. 1992. Selenocysteyl-tRNAs recognize UGA in *Beta vulgaris*, a higher plant, and in *Gliocladium virens*, a filamentous fungus. *Biochem. Biophys. Res. Comm.* 184:254-259.

Hassan, M., Sinden, S. L., Kobayashi, R. S., Nordeen, R. O., and Owens, L. D. 1993. Transformation of potato (*Solanum tuberosum*) with a gene for an anti-bacterial protein, cecropin. *Acta Hort.* 336:127-131.

Sinden, S. L., Kobayashi, R. S., Nordeen, R. O. and Owens, L. D. 1993. Perspectives on controlling potato soft rot with foreign genes for antibacterial compounds. *Acta Hort.* 336:79-83.

Wozniak, C. A. and Owens, L. D. 1993. Use of β -glucuronidase (GUS) as a marker for transformation in sugarbeet. *J. Sugar Beet Research* 30:299-316.

Wozniak, C. A. and Owens, L. D. 1994. Native β -glucuronidase activity in sugarbeet (*Beta vulgaris*). *Physiol. Plant.* 90:763-771.

Gercheva, P., Zimmerman, R. H., Owens, L. D., Berry, C., and Hammerschlag, F. A. 1994. Particle bombardment of apple leaf explants influences adventitious shoot formation. *HortScience* 29:1536-1538.

Mills, D., Hammerschlag, F. A., Nordeen, R. O., and Owens, L. D. 1994. Evidence for the breakdown of cecropin B by proteinases in the intercellular fluid of peach leaves. *Plant Sci.* 104:17-22.

Symposia, Book Chapters and Proceedings

Hammerschlag, F. H., Zimmerman, R. H., and Owens, L. D. 1993. Eds. "Second International Symposium on In Vitro Culture and Horticultural Breeding" *Acta Hort.* 336, 404 pp.

Owens, L. D. 1995. Strategies for developing horticulturally useful genes: Overview of gene availability, identification and regulation. *HortScience*, In press.

Manuscripts Submitted for Publication

Wozniak, C.A., and Owens, L.D. Metal catalysis of glucuronide-based substrates mediated by tungsten, Cu^{+2} , Fe^{+2} , and Zn^{+2} .
Physiol. Plant., 6/94.

I. Name: Freddi A. Hammerschlag
Title: Research Plant Physiologist

II. CRIS Project: 1275-21000-077-00D
CRIS Title: GENE TRANSFER AND TISSUE CULTURE TECHNOLOGIES
FOR IMPROVEMENT OF PEACH, APPLE, PEAR, AND
SOYBEAN

Objective 1: Develop techniques for gene transfer to improve disease resistance and morphogenesis in peach.

Progress: Peach transformants containing a cytokinin-specifying *ipt* gene from *Agrobacterium tumefaciens* were shown to grow and multiply similarly to compact peach trees on selective medium. Under greenhouse conditions, two of the three transformants have exhibited a more compact growth habit when compared to nontransformants. A protocol for producing peach protoplasts was developed and the effects of the bacteriocidal peptide cercropin B on peach protoplasts, a suspension of leaf mesophyll cells and cells of intact leaves were determined. These studies established that there is a wide range of concentrations at which cecropin B will kill intruding bacteria before causing any harm to the peach cells and although as much as 90% of the activity of cecropin B could be destroyed by the intercellular fluid in 10 min, levels adequate for lethality against bacterial pathogens remain. These results provide evidence for the feasibility of introducing cecropin into peach to enhance resistance against bacterial pathogens. An in vitro micrografting technique, utilizing a digoxigenin (DIG)-labeled cRNA probe derived from *Prunus* necrotic ringspot virus (PNRSV) RNA 3, was developed to monitor PNRSV in peach shoot cultures. This method will be useful for screening transformed peach shoots for resistance to PNRSV.

Plans: We will continue to investigate the stability of the *ipt* gene in peach transformants. Tissue culture techniques will be used to isolate pure transformants from those that are chimeric in nature. Studies will be conducted to improve the efficiency of peach transformation via *Agrobacterium*-mediated gene transfer and via particle gun bombardment. Since plant regeneration is the major limiting step in the production of transformants, studies will be conducted to determine conditions for inducing regeneration from embryogenic callus 3-12 months after peach embryo isolation and from explants derived from peach cultivars maintained in vitro. Experiments will be conducted to introduce the cecropin gene into peach and to compare the efficiency of several transformation vectors containing the coat protein gene of PNRSV.

Objective 2. Determine the frequency, nature and stability of somaclonal variation in peach regenerants.

Progress: Field trials and bioassays have established heritabil-

ity of bacterial spot (*Xanthomonas campestris* pv. *pruni*) resistance in peach somaclones. Response of somaclones to several strains of *X. campestris* pv. *pruni* has also been determined. Somaclonal variants with increased tolerance to *Meloidogyne incognita* have been identified and evaluated. The frequency of somaclonal variation was shown to be genotype dependent and a genetic basis for somaclonal variation has been established.

Plans: Peach somaclones and progeny with high levels of leaf spot resistance will be propagated in vitro, grafted onto peach short life resistant rootstock BY520-9 and evaluated under field conditions.

Objective 3. Develop techniques for transferring genes into apple and regenerating transgenic plants.

Progress: Conditions for high frequency regeneration of apple shoots following particle bombardment have been established. Studies with antibiotics reveal that cefotaxime at 100 µg/ml inhibits regeneration, whereas a similar level of carbenicillin stimulates regeneration.

Plans: Studies will be conducted to determine conditions for eliminating *Agrobacterium tumefaciens* following cocultivation without negatively impacting plant regeneration. Because apple stems are more readily infected with *Agrobacterium* than leaves, but plant regeneration occurs more readily from leaves than from stems, experiments will be conducted to increase the efficiency of regeneration from apple stems and the efficiency of infection on apple leaves. Transformation studies will be conducted to introduce the cecropin gene into apple and to evaluate whether this gene protects apple against infection with *Erwinia amylovora*.

III. Cooperators:

- Rose Hammond, MPPL, BARC
- Richard Zimmerman, FL, BARC
- Robin Huettel, USDA/APHIS, Hyattsville, MD
- Ghazala Hashmi, Rutgers University
- Tim Ng, Univ. of Md., College Park
- Pam Healey, Univ. of Md., College Park
- Ghazala Hashmi, Rutgers University
- David Ritchie, NC State University
- Dennis Werner, NC State University
- Ralph Scorza, USDA/ARS, Kearneysville, WV
- Petya Gercheva, Fruitgrowing Institute, Bulgaria
- Vladislav Ognjanov, Inst. of Viticulture, Fruitgrowing and Horticulture, Yugoslavia
- Bruno Mezzetti, Univ. of Ancona, Italy

•David Mills, Ben Gurion University, Israel

IV. Publications (since last review)

Peer Reviewed

Mezzetti, B., Zimmerman, R. H., Mischke, C. and Hammerschlag, F. A. 1992. Merocyanine 540 as an optical probe to monitor the effects of culture filtrates of *Phytophthora cactorum* on apple cell membranes. *Plant Science* 83:163-167.

Crosslin, J. M., Hammond, R. W. and Hammerschlag, F. A. 1992. Detection of *Prunus* necrotic ringspot virus in herbaceous plants and *Prunus* species using a cRNA probe. *Plant Disease* 76:1132-1136.

Mezzetti, B., Rosati, P., Zimmerman, R. H. and Hammerschlag, F. A. 1993. Determination of resistance to *Phytophthora cactorum* culture filtrate in apple clonal rootstocks, cultivars and leaf regenerants, using in vitro proliferation and optical probe methods. *Acta Hort.* 336:93-99.

Ritchie, D. F., Werner, D. J. and Hammerschlag, F. A. 1993. Field evaluation of tissue culture-derived peach trees for susceptibility to bacterial spot (*Xanthomonas campestris* pv. *pruni*). *Acta Hort.* 336:155-163.

Huettel, R. N. and Hammerschlag, F. A. 1993. Response of peach scion cultivars and rootstocks to *Meloidogyne incognita* in vitro and in microplots. *J. Nematology* 25:472-475.

Mills, D. and Hammerschlag, F. A. 1993. Effect of cecropin B on peach pathogens, protoplasts, and cells. *Plant Sci.* 93:143-150.

Mills, D. and Hammerschlag, F. A. 1994. Isolation of cells and protoplasts from leaves of in vitro propagated peach (*Prunus persica*) plants. *Plant Cell Tissue & Organ Cult.* 36:99-105.

Healey, P., Ng, T. and Hammerschlag, F. A. 1994. Response of leaf spot sensitive and tolerant muskmelon (*Cucumis melo* L.) mesophyll and suspension cells to the phytotoxin roridin E. *Plant Sci.* 97:15-21.

Mackay, W. A., Ng, T. and Hammerschlag, F. A. 1994. *Cucumis melo* response to toxins produced by *Myrothecium roridum* Tode ex. Fries. *J. Amer. Soc. Hort. Sci.* 119:356-360.

Hammerschlag, F. A., Werner, D. J. and Ritchie, D. F. 1994. Stability of bacterial leaf spot resistance in peach regenerates under in vitro, greenhouse and field conditions. *Euphytica* 76: 101-106.

Hashmi, G., Huettel, R., Hammerschlag, F. A. and Krusberg, L. 1994. Optimum levels of *Meloidogyne incognita* inoculum for infection of tomato and peach under in vitro conditions. J. Nematology. (In press)

Gercheva, P., Zimmerman, R. H., Owens, L. D., Berry C., and Hammerschlag, F. A. 1994. Particle bombardment of apple leaf explants influences adventitious shoot information. HortScience. 29:1536-1538.

Hammerschlag, F. A., Ritchie, D., Werner, D., Hashmi, G., Krusberg, R., Meyer, R., and Heuttel, R. 1995. In vitro selection of disease resistance in fruit trees. Acta Hort. (In press)

Mills, D., Hammerschlag, F. A., Nordeen, R. O., and Owens, L. D. 1995. Evidence for the breakdown of cecropin B by proteinases in the intercellular fluid of peach leaves. Plant Sci. (In press)

Mezzetti, B., Capasso, R., Evidente, A., Hammerschlag, F. A., Zimmerman, R. H., Cristinzio, G., and Rosati, P. 1995. Interaction of partially purified phytotoxins from *Phytophthora cactorum* on apple cell plasma membranes. J. Phytopathol. (In press)

Symposia, Book Chapters, and Proceedings

Hammerschlag, F. A. and Litz, R. E. (eds.) 1992. Biotechnology of Perennial Fruit Crops. C. A. B. International, Wallingford.

Hammerschlag, F. A. 1992. Somaclonal Variation, pp. 35-55. In: Biotechnology of Perennial Fruit Crops. F. A. Hammerschlag and R.E. Litz (eds.). C. A. B. International, Wallingford.

Scorza, R. and Hammerschlag, F. A. 1992. Stone fruits, pp. 277-301. In: Biotechnology of Perennial Fruit Crops. F. A. Hammerschlag and R. E. Litz (eds). C. A. B. International Wallingford.

Hammerschlag, F. A., Zimmerman, R. H., and Owens, L. D. 1993. Eds. "Second International Symposium on In Vitro Culture and Horticultural Breeding," Acta Hort. 336, 404 pp.

Scorza, R., Hammerschlag, F. A., Zimmerman, T. W. and Cordts, J. M. 1995. Transformation in peach and plum, pp. In: Biotechnology in Agriculture and Forestry. Springer-Verlag, Berlin (In press)

Manuscripts Submitted for Publication:

Heuss-La Rosa, K., Hammond, R., Crosslin, J. M., Hazel, C. and Hammerschlag, F. A. 1995. Monitoring Prunus necrotic ringspot virus infection by hybridization with a cRNA probe following in vitro micrografting.

Hashmi, G. P., Krusberg, L. R., Huettel, R. N. and Hammerschlag, F. A. 1995. Growth, development and response of peach somaclones to the root-knot nematode.

I. Name: Peter P. Ueng
Title: Research Plant Pathologist

II. CRIS Project: 1275-21220-023-00D
CRIS Title: IDENTIFICATION AND CHARACTERIZATION OF
DISEASE RESISTANCE GENES AND GENE PRODUCTS OF
WHEAT

Objective 1: Molecular tools to identify *Septoria* diseases in cereals.

Progress: Five multiple sequence clones isolated from *Septoria tritici* genomic DNA and four specific primer set sequences selected for DNA amplification of *Stagonospora nodorum* have been established.

Plans: Pathogenesis of *Septoria* fungi in diseased plants will be studied by dot blot hybridization (*S. tritici*) and PCR amplification (*S. nodorum*).

Objective 2: Restriction fragment length polymorphisms (RFLP) technique to study genetic variations of *Septoria* fungal pathogens in cereals and common grasses.

Progress: DNA probes that can distinguish barley-biotype *S. nodorum*, wheat-biotype *S. nodorum* and *S. avenae* f.sp. *triticea* were developed. *S. nodorum* has less genetic variation, while *S. a. triticea* has much more. Barley-biotype *S. nodorum* is not phylogenetically closely related to wheat-biotype *S. nodorum*, and can not attack wheat. On the contrary, some wheat-biotype *S. nodorum* can attack both wheat and barley.

Plans: To understand the host specificity of *Septoria* diseases, barley-biotype *S. nodorum* will be transformed with DNA fragments from wheat-biotype *S. nodorum*. The transformed isolates will be used for pathogenicity test to identify virulence determinant(s) in *S. nodorum* against wheat.

Objective 3: Characterizing ethylene-synthesis genes in wheat.

Progress: Two cDNA clones of ACC synthase gene have been isolated by PCR amplification of messenger RNA from etiolated wheat seedlings using the conserved sequence information of the genes from other plant species as primers. The genes have been partially sequenced.

Plans: Study the regulation of ACC synthase genes in diseased plants. Transform the gene in antisense orientation into wheat so that the transgenic wheat produces less ethylene during pathogen-

esis. Delaying tissue senescence may sustain or increase plant resistance toward fungal, bacterial and viral diseases while retaining yield.

Objective 4: Identification of disease resistant gene(s) in wheat against *S. tritici*.

Plans: The disease resistant gene(s) will be identified by RFLP comparison of resistant and susceptible cultivars and by subtractive hybridization in combination of PCR amplification.

III. Cooperators

- Gary C. Bergstrom, Cornell University
- Greg Shaner, Purdue University
- Barry M. Cunfer, University of Georgia
- Weidong Chen, University of Illinois

IV. Publications (since last review)

Peer Reviewed

Ueng, P. P., Vincent, J. R., Kawata, E. E., Lei, C. H., Lister, R. M., and Larkins, B. A. 1992. Nucleotide sequence analysis of the genomes of the MAV-PS1 and P-PAV isolates of barley yellow dwarf virus. *J. Gen. Virol.* 73:487.

Ueng, P. P., Bergstrom, G. C., Slay, R. M., Geiger, E. A., Shaner, G., and Scharen, A. L. 1992. Restriction fragment length polymorphisms in the wheat glume blotch fungus, *Phaeosphaeria nodorum*. *Phytopathology* 82:1302.

Ueng, P. P. and Chen, W. 1994. Genetic differentiation between *Phaeosphaeria nodorum* and *P. avenaria* using restriction fragment length polymorphisms. *Phytopathology* 84:800.

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I. Name: Eliot M. Herman
Title: Plant Physiologist

II. CRIS Project: 1275-21000-047-00D
CRIS Title: THE REGULATION AND EXPRESSION OF GENES
INVOLVED IN SEED OIL AND PROTEIN PRODUCTION

Progress: The genomic sequences and cDNAs encoding soybean oleosin, thiol protease and BiP have been characterized. The synthesis and processing of the thiol protease was analyzed. This protease likely functions in processing and assembly of seed storage proteins. The molecular regulation of coordination of the two agriculturally important soybean storage products is unknown and has considerable significance for the modification of the ratio of protein and oil in soybean seeds. It is known that oil and protein accumulation is genetically linked. Genes for storage proteins and oil body proteins share common controlling elements. This may provide a molecular basis that links the accumulation of protein and oil.

Plans: The characterization of the control of storage protein and oil body protein remains to be completed. Embryogenic soybean cultures could be used to look for common and different regulatory elements and controls.

This CRIS Project was abolished in FY '94.

CRIS Project No. 1275-21000-112-00D
CRIS Title: CELLULAR AND MOLECULAR ASPECTS OF PROMPT
AND ADAPTIVE RESPONSES TO ENVIRONMENTAL
STRESS IN WHEAT

Progress: This is a recent new redirection and the research plans are in progress.

Plans: A new project statement will be developed and submitted for approval. The proposed research program will examine the cellular and molecular aspects of the prompt and adaptive responses of wheat plants to environmental stress. Initial research emphasis will be directed toward characterizing the stress response of the endomembrane system to reduce cellular damage. A multidisciplinary approach using molecular, biochemical, and structural techniques will be used.

CRIS Project No. 1275-21000-047-09R (BARD)

CRIS Title: RECOGNITION AND OF MISFOLDED SEED PROTEINS

Progress: This project's objectives are to elucidate the mechanism by which plant cells identify and degrade proteins synthesized in transgenic plants. Current progress has identified the vacuole as the intracellular site where unstable engineered proteins are selectively degraded.

Plans: The protease that mediates the selective degradation of malformed proteins expressed in transgenic plants will be identified and cloned. An antisense expression of the protease may allow the unstable protein to be accumulated. Other studies will change the intracellular targeting of the misfolded and normal protein in order to develop alternate strategies to improve the stability of engineered proteins.

III. Cooperators

- B. Yaklich, SARL, BARC
- Shyam Dwivedi, Howard University
- Heven Sze, Univ. of Md., College Park
- Chris Lamb, Salk Institute
- Maarten Chrispeels, UC, San Diego
- Russell Jones, UC, Berkeley
- Alan Bennett, UC, Davis
- John Ohlrogge, Michigan State University
- Anthony Kinney, Dupont, DE
- Alan Jones, University of North Carolina
- Gadi Galili, Weizmann Institute, Israel
- T. Minimakawa, Tokyo Metropolitan University, Japan
- Denis Murphy, John Innes Institute, UK

IV. Publications (since last review):

Peer reviewed

Herman, E. M. and Lamb, C. J. 1992. Arabinogalactan-rich glycoproteins are localized on the cell surface and in intravacuolar multivesicular bodies. *Plant Physiol.* 98: 264-272

Kalinski, A. J., Melroy, D. L., Dwivedi, R. S., and Herman, E. M. 1992. A soybean vacuolar protein (P34) related to thiol proteases which is synthesized as a glycoprotein precursor during seed maturation. *J. Biol. Chem.* 267: 12068-12076

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Jones, A. M. and Herman, E. M. 1993. A K-D-E-L-containing auxin-binding protein is located at the plasma membrane and within the cell wall. *Plant Physiol.* 101: 595-606

Loer, D. S. and Herman, E. M. 1993. Cell-free synthesis of the oil body membrane protein oleosin: specific cotranslational integration into microsomal membranes. *Plant Physiol.* 101: 993-998

Lui, D., Li, N., Kalinski, A., Dube, S., Herman, E. M., and Mattoo, A. K. 1993. Molecular characterization of a rapidly wound-induced soybean (*Glycine max* L.) gene encoding 1-aminocyclopropane-1-carboxylate synthase. *Plant Cell Physiol.* 34: 1151-1157

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Sikora, L. J., Kuykendall, L. D., Dwivedi, R. S., Herman, E. M., and Enkiri, N. K. 1994. Characterization of a 14 kDa component with low expression in a unique Nod⁺Fix⁻ *Bradyrhizobium japonicum*. *Microbiology*, 140:2761-2767.

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Pueyo, J. J., Chrispeels, M. J. and Herman, E. M. 1995. Degradation of a secretory protein with a destabilizing epitope occurs in the vacuole. *Planta*, (in press).

Yaklich, B. and Herman, E. M. 1995. Protein storage vacuoles of soybean aleurone cells accumulate a unique glycoprotein as well as proteins thought to be embryo specific. *Plant Science* (accepted Jan. 9, 1995).

Symposia, Book Chapters and Proceedings:

Herman, E. M. 1992. Genetically engineered seeds. *Audubon Naturalist News* 18: 5-7.

Herman, E. M. 1994. Multiple origins of intravacuolar protein accumulation of plant cells. Adv. in Structural Biology 3: 243-283.

Herman, E. M. 1994. The cell and molecular biology of seed oil bodies. In; Seed Development and Germination. Ed. J. Kigel, G. Gallili., M. Dekker Inc., pp 195-214.

I. Name: Ann C. Smigocki
Title: Research Geneticist

II. CRIS Project: 1275-21000-079-00D
CRIS Title: GENE TRANSFER TO SUGARBEET AND TOMATO FOR
IMPROVED CARBON PARTITIONING AND PEST
RESISTANCE

Objective 1: Influencing plant growth and development by *in vivo* manipulation of hormone ratios in transgenic plants carrying reconstructed cytokinin biosynthesis genes.

Progress: A genetically modified bacterial gene encoding the first enzyme in the cytokinin biosynthetic pathway is being utilized to study the mode of action of cytokinin in control of growth and development in transgenic tomato, tobacco and peach plants. To allow for temporal and spatial regulation of overexpression of the cytokinin gene *in planta*, promoters from genes known to be switched on by developmental, physical, and/or chemical factors were fused with the gene's coding region. Targeting of transient cytokinin overproduction by a heat shock promoter induced some characteristic cytokinin effects such as reduced height, release of axillary buds, and greening of leaves but did not completely inhibit root growth as did constitutive overproduction of cytokinin, thus facilitating regeneration of whole plants. When the cytokinin gene was fused to a wound inducible promoter, characteristics not previously associated with the expression of this gene were observed. Transgenic plants exhibited early bolting, pronounced apical dominance, increased height and leaf size, and reduced chlorophyll content. However, whole plant senescence was delayed by up to a few months and was correlated with induction of root-specific production of cytokinin upon cessation of plant growth. Application of this technology to economically important crops may prove to be of agronomic significance for increasing productivity through extension of the growth period since field applications of cytokinins are not cost effective due to their instability and high cost.

Plans: Despite reports of similar enzymatic activity in both plants and bacteria, the endogenous plant cytokinin biosynthesis gene has not been cloned. Cloning of this gene would facilitate manipulation of plant physiological processes using genetic engineering approaches. We plan to determine the degree of homology between the bacterial and plant endogenous cytokinin biosynthesis gene by antisense technology. Further characterization and molecular analysis of these plants may potentiate the cloning of the plant gene.

Objective 2: Effects of transient overproduction of cytokinins on modulation of gene expression during early shoot development in transgenic plants.

Progress: We determined that transient overproduction of cytokinins in plants transformed with the bacterial cytokinin biosynthesis gene specifically up-regulated a small subset of genes that more accurately reflected changes in gene expression in response to *in vivo* cytokinin fluctuations. Establishing the kinetics of induction of cytokinin-regulated mRNAs by *in vitro* translation assays facilitated construction of a cDNA library enriched for genes modulated by cytokinin. Screening of the library with a subtracted probe yielded numerous cDNA clones that represent approximately 60 groups of genes by cross hybridization analysis. Sequencing a cDNA clone from each representative group identified three classes of genes. One class of cDNA clones shared strong sequence similarities to nuclear-encoded chloroplast genes some of which have previously been shown to be regulated by cytokinins. Another set of clones corresponded to genes that may be induced as a general response to stress suggesting that cytokinin- and stress-related genes may be regulated by both influences and that cytokinin may be involved in coordination of growth with changing environmental conditions. We have also isolated clones that do not appear to share sequence homologies with any known genes.

Plans: We will isolate genomic sequences using specific cDNA clones and identify promoter regions for determining cytokinin's role in signal transduction pathways. The cDNAs will be cloned into *E. coli* expression vectors for protein purification and antibody production.

Objective 3: The role of elevated endogenous cytokinin levels on induction of natural plant defense mechanisms.

Progress: Since the present focus for crop improvement is to combine natural defense mechanisms of plants and biotechnology, we evaluated cytokinin's role as a modulator of insect resistance in transgenic *Nicotiana plumbaginifolia* plants carrying a wound-inducible cytokinin biosynthesis gene. Enhanced resistance was observed to a number of insects, including the herbivorous pest, tobacco (tomato) hornworm and a virus-transmitting pest, the green peach aphid. When leaf material or whole plants were infested with the tobacco hornworm larvae, a significant reduction in consumption of leaves was observed as compared to the controls. The effect on the green peach aphid nymphs was more dramatic. Only approximately half of the newly hatched nymphs reached adulthood and of those only a small percentage were able to reproduce. The level of insect tolerance in transgenic plants was further enhanced by boosting the endogenous cytokinin concentrations with exogenous zeatin applications that had proved ineffective in non-transformed plants.

Plans: Presently, little is known about the mode of action of the cytokinin biosynthetic gene on enhanced disease resistance, but involvement of secondary metabolites is suspected. Bioassays of fractionated plant extracts for insecticidal activity will aid in identifying any compound(s) that may be involved in natural plant defense properties and the corresponding biosynthetic pathway(s) that are modulated by cytokinins.

Objective 4: Determine cytokinin's participation in carbon partitioning in tomato and sugar beet plants transformed with reconstructed cytokinin-biosynthesis genes.

Progress: Since numerous studies have demonstrated that application of cytokinins to plant organs causes the transport of assimilates towards that site, such studies will be done in transgenic plants carrying reconstructed cytokinin biosynthesis genes. The mechanism behind this transport is not known but local accumulation of cytokinin in sink organs such as seeds, fruits, and roots may lead to increased assimilate transport to those organs and increased plant productivity. Transgenic plants carrying the cytokinin gene constructs have been regenerated and, in a collaborative effort with Dr. L. D. Owens, similar experiments have been initiated with sugar beet. Since phytohormone status appears to determine the developmental changes in the sugar beet storage root, this approach has the potential to increase the sugar content of the taproot.

Plans: Determine the sugar content in the fruit and taproot of transgenic tomato and sugar beet plants, respectively. Assay the activities of sucrose-metabolizing enzymes, as well as levels of expression of genes encoding these enzymes, and the distribution of radioactive assimilates in transgenic plants before and after cytokinin induction.

III. Collaborators:

- Gary Baughan, SARL, BARC
- George Buta, HCQL, BARC
- Tom Elden, SARL, BARC
- Ed Lee, CSL, BARC
- Carol Auer, University of Connecticut
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- Sherry Kitto, University of Delaware
- Dennis Schaff, University of Delaware
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- Radomir Konjevic, Inst. for Biological Research, Belgrade, Yugoslavia
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- German Spangenberg, Institute for Plant Sciences, Zurich, Switzerland
- Christopher Downs, Food and Crop Research, Levin, New Zealand

IV. Publications (since last review):

Peer Reviewed

Smigocki, A. C., Neal, J. W., McCanna, I. J., and Douglass, L. 1993. Cytokinin-mediated insect resistance in *Nicotiana* plants transformed with the *ipt* gene. *Plant Mol. Biol.* 23:325-335.

Harding, S. A. and Smigocki, A. C. 1994. Cytokinins modulate stress response genes in *ipt*-transformed *Nicotiana plum-baginifolia* plants. *Physiol. Plant.* 90:327-333.

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Smigocki, A. C. 1995. Phenotype modification and enhanced insect resistance mediated by regulated expression of the cytokinin biosynthesis gene. *HortScience* (in press).

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Berry, C., Van Eck, J. M., Smigocki, A. C., and Kitto, S. L. 1994. *Agrobacterium*-mediated transformation of commercial mints.

**Patents**

Smigocki, A. C. 1993. Enhanced insect resistance in plants genetically engineered with a plant hormone gene involved in cytokinin biosynthesis. Patent Serial No. 08/054,985; Docket No. 0175.92; Filed April 30, 1993.



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Reigold, A. C. 1953. Enhanced insect resistance in plants
genetically engineered with a plant hormone gene involved in
cytokinin biosynthesis. Patent Series No. 02/054,753; Doct. No.
0175.93; Filed April 20, 1953.